Commentary

Development and evolution occlude: Evolution of development in mammalian teeth

P. David Polly*

Molecular and Cellular Biology Section, Division of Biomedical Sciences, Queen Mary & Westfield College, London E1 4NS, United Kingdom

According to Roy Lewis in *The Evolution Man*, an evolving mammal worries about nothing more than it does its teeth (1). And to a paleontologist, nothing about a mammal matters more than its teeth. To paleontologists, teeth are the population markers that microsatellite sequences are to population biologists. Even when represented by only a single tooth, a fossil mammal can often be identified by cusp number, relative cusp positions, and cusp heights, all features that are unfathomable to the non-paleontologist. But in this issue of PNAS, Jernvall *et al.* (2) penetrate the fascinating "lost world" of fossil mammals, blazing a new path connecting molecular developmental biology with paleontology. Recent work, much of it by these same researchers, has given us a general framework for understanding the molecular basis of tooth crown formation (3–5), integrating genes and proteins into our previous histology-based knowledge of tooth development (6–8). It is now well known that enamel knots—transient structures that appear first at the apex of the developing tooth bud and later at the apices of growing cusps—are important molecular signaling centers that control the placement and size of individual cusps on the crown (4, 9). Enamel knots, via a cascade of gene activity, regulate their own integrity, stimulate growth in surrounding epithelium, induce the formation of subsequent knots (in some cases), and signal their own demise via apoptosis (Fig. 1). As molecular signaling centers, enamel knots seem to be a universal aspect of mammalian tooth crown patterning. They have been found in all species examined to date, regardless of tooth morphology. Their discovery has helped explain the mechanism of crown patterning but does not, by itself, explain why mammals have such a diversity of teeth. As common features, a shared gene cascade can explain only the similarities among species, not their differences.

The paper by Jernvall *et al.* in this issue of PNAS (2) takes an important step toward explaining differences. Even though they shared an ancestor about 20 million years ago, mice and voles have quite different teeth. Mouse molars, typ-ical of murine rodents, have rounded pairs of cusps arranged in transverse rows. Vole molars have alternating triangular cusps arranged in a zigzag pattern down the length of the crown, which looks very much like a Christmas tree. Vole teeth also have more cusps than mice, and the rapid evolutionary multiplication of those cusps has made voles important biostratigraphic markers for determining the age of Quaternary faunas (10, 11). Despite the morphological differences, Keränen *et al.* (12) found in 1998 that mice and voles share many aspects of the development of their teeth.

Now, however, Jernvall *et al.* have spotted differences in gene expression in the developing tooth bud that seem to explain the disparity in adult tooth morphology. They first looked at the spatial pattern of expression of *Fgf4*, *Lef1*, *p21*, and *Shh* genes, all of which were known to function in different signaling pathways. They quantified the changing spatial patterns of gene expression and developing morphology by using geographic information system technology. This allowed them to calculate cause-and-effect correlations between gene activity at one embryonic stage with morphological differentiation at later stages. They found first that expression prepatterns exist before morphological differentiation has begun, and that the spatial distribution of gene activity reflects the subsequent location of cusps. In mice, the molecular signaling center associated with the protoconid cusp is shifted anteriorly so that it lies next to the metaconid prepatterning center. This results in the transversely paired cusps characteristic of mice. In voles, the protoconid center lies at the posterior end of the primary enamel knot, offset diagonally from the metaconid center. This produces the offset between the cusps characteristic of voles. It is clear that the between-species difference is set up early in tooth development, before the cusps begin to form, not as a result of postformation growth.

The second thing Jernvall *et al.* found was that the multiplication of cusps in vole teeth is associated with a different developmental process. During crown morphogenesis, vole tooth buds grow anteroposteriorly at a much faster rate than mouse buds. The growth is accompanied by repeated iteration of the offset cusp pattern. This produces a long tooth with a zigzag arrangement of many triangular cusps. Vole teeth are probably the most variable of any living mammal (13), which may be explained by variation in the rate of growth and cusp iteration found by Jernvall *et al.* Thus, two possibly independent changes took place in the evolutionary differentiation of mice and vole molar morphology. In the mouse lineage, the early spatial pattern of gene expression was altered to produce a parallel cusp arrangement; in the vole lineage, the offset pattern was not changed, but the package was reiterated multiple times down the length of a quickly growing bud. Paleontologists have long known the evolutionary sequence of morphological changes leading to the differences in mice and vole molars and many of the ecological and dietary shifts accompanying their divergence (14, 15). Now, for the first time, Jernvall *et al.* have given us insight into the molecular basis of changes in the developmental system necessary to accomplish that evolution.

This is exciting stuff. Jernvall *et al.* have taken us from a static view of shared gene expression pathways to an evolutionarily dynamic view in which patterns of expression are modified with descent. This is the same shift in perspective that Darwin's evolution brought to Aristotelian typology. Some evolutionary developmental biologists see themselves in opposition to traditional population genetics, with its notions of variation and selection (16). But Jernvall *et al.*'s work contains the rudimentary aspects of within- and between-species analysis of variation, the trademark of quantitative genetics (for a similar perspective, see refs. 17 and 18). The exciting part of this study is that, unlike most developmental research, it has a handle on variation, but, unlike classic population genetics, it analyzes genes, gene products, developmental in-
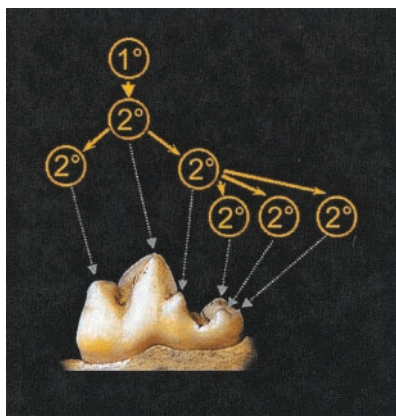
COMMENTARY

Fig. 1. The relationship between the enamel knot cascade in mammalian tooth crown morphogenesis and the topography of cusps on an erupted dog (*Canis familiaris*) lower first molar. In development, the tooth crown topography is initiated by the primary enamel knot (1°). This begins a cascade of gene expression, ultimately resulting in secondary enamel knots (2°), one for each major cusp on the tooth crown. The spatial placement of knots in the developing tooth bud corresponds to the topographical relationship of the cusps, and the timing of the knots is related to cusp heights (9).

teractions, and morphologic traits together. And, importantly, this study deduces the historical sequence of changes to the developmental system by using a comparative phylogenetic framework, including paleontological data. This is not just evolutionary developmental biology; this is evolutionary biology full stop. There is still a long way to go, however. The paper by Jernvall *et al.* looks only at a handful of individuals representing two species. It will be interesting to see what sort of population polymorphism there is in the genes involved in tooth morphogen-

esis. Working on inherited clinical conditions, some researchers have identified developmental genetic differences between individuals with and without the presenting symptoms (19–21). Others have looked at population-level morphological variation in the context of molecular developmental processes (22–24). However, the day will soon arrive when a true survey of population variation in patterns of developmental gene expression is possible. The spatial analysis introduced by Jernvall *et al.* will be an important tool for that study.

Teeth are becoming *the* model organ system for studying mechanisms of evolution. A model organ system differs from a model organism because it allows comparisons between species or larger taxonomic groups; truly integrative approaches to evolution lie in those comparisons. Advances in the molecular developmental genetics of tooth morphogenesis would not be enough to make evolutionary dental research so attractive; however, research on a variety of dental topics is providing new opportunities for synthesis. The allometry between molar size and body mass allows the estimation of body mass in fossil species and, by proxy, estimation of mass-specific reproductive and physiological parameters such as generation time, metabolic rate, and longevity (25–27). The sequence of tooth formation and eruption in dentition appears to be related to postnatal growth patterns and maturation, opening the possibility of reconstructing life histories for extinct species (28, 29). Enamel microstructure reveals information about the development (30) and function (31, 32) of teeth and gives clues to the phylogenetic relationships of species (33, 34). Incremental lines

in enamel and dentine provide clues to individual growth and life history (35, 36), and stable isotopes extracted from those tissues give direct evidence of environmental and physiological parameters from the life history of individual organisms (37, 38). New quantitative techniques show there is considerable variety in the tempo and mode of dental evolution, but that it behaves in accordance with established models of evolutionary change (39, 40). And, intriguingly, mitochondrial DNA has now been extracted from Quaternary fossil teeth, allowing fossil samples to be placed in the same population genetic framework as extant populations living in the same geographic area (41). The sheer richness of information associated directly with teeth makes them a growth area for integrative evolutionary research. Partial syntheses of tooth morphology, microstructure, diet, ecology, and evolution have already been made (42, 43). We should expect to see this trend continue.

Jernvall *et al.* set a high standard for evolutionary developmental studies. They have seamlessly connected gene activity, developmental processes, and adult morphology. They have applied quantitative testable statistics to the study of gene expression and its correlation with morphogenesis. And they have framed their developmental work in a comparative perspective, asking not only what genes are active in morphogenesis but also how gene expression differs when morphologies are not the same. Jernvall *et al.* have brought together the evolution and molecular developmental biology of teeth in the same complex multifunctional way that opposing teeth come together in occlusion.

1. Lewis, R. (1993) *The Evolution Man, or, How I Ate My Father* (Pantheon, New York).
2. Jernvall, J., Keränen, S. V. E. & Thesleff, I. (2000) *Proc. Natl. Acad. Sci. USA* **97**, 14444–14448.
3. Maas, R. & Bei, M. (1997) *Crit. Rev. Oral Biol. Med.* **8**, 4–39.
4. Jernvall, J., Åberg, T., Kettunen, P., Keränen, S. & Thesleff, I. (1998) *Development (Cambridge, U.K.)* **125**, 161–169.
5. Jernvall, J. & Thesleff, I. (2000) *Mech. Dev.* **92**, 19–29.
6. Butler, P. M. (1956) *Biol. Rev.* **31**, 30–70.
7. Gaunt, W. A. (1961) *Acta Anat.* **45**, 219–251.
8. Butler, P. M. (1995) *Int. J. Dev. Biol.* **39**, 25–34.
9. Jernvall, J. (1995) *Acta Zool. Fenn.* **198**, 1–61.
10. Repenning, C. A. (1987) in *Cenozoic Mammals of North America: Geochronology and Biostratigraphy*, ed. M. O. Woodburne (Univ. of California Press, Berkeley, CA), pp. 236–268.
11. Bell, C. J. (1998) in *Dating and Earthquakes: Review of Quaternary Geochronology and Its Application to Paleoseismology*, eds. Sowers, J. M., Noller, J. S. & Lettis, W. R. (Nuclear Regulatory Comm., Washington, DC), NUREG/CR No. 5562, pp. 2-605–2-645.

12. Keränen, S. V. E., Åberg, T., Kettunen, P., Thesleff, I. & Jernvall, J. (1998) *Dev. Genes Evol.* **208**, 477–486.
13. Bell, C. J. & Repenning, C. A. (1999) *J. Vert. Pal.* **19**, 757–766.
14. Guthrie, R. D. (1965) *Evolution (Lawrence, KS)* **19**, 214–233.
15. Jacobs, L. L. & Downs, W. R. (1994) in *Rodent and Lagomorph Families of Asian Origins and Diversification*, National Science Museums Monographs, eds. Tomida, Y., Li, C. K. & Setoguchi, T. (National Science Museums Monographs, Tokyo), Vol. 8, pp. 149–156.
16. Gilbert, S. F., Opitz, J. M. & Raff, R. A. (1996) *Dev. Biol.* **173**, 357–372.
17. Ahn, D. & Gibson, G. (1999) *Dev. Genes Evol.* **209**, 473–481.
18. Palopoli, M. F. & Patel, N. H. (1996) *Curr. Opin. Genet. Dev.* **6**, 502–508.
19. Kere, J. Srivastava, A. K., Montonen, O., Zonana, J., Thomas, N., Ferguson, B., Munoz, F., Morgan, D., Clarke, A., Baybayan, P., *et al.* (1996) *Nat. Genet.* **13**, 409–416.
20. Vastardis, H., Karimbux, N., Guthua, S. W., Seidman, J. G. & Seidman, C. E. (1996) *Nat. Genet.* **13**, 417–421.

21. Mundlos, S. (1999) *J. Med. Genet.* **36**, 177–182.
22. Jockusch, E. L. (1997) *Evolution (Lawrence, KS)* **51**, 1966–1982.
23. Polly, P. D. (1998) *Paleobiology* **24**, 409–429.
24. Jernvall, J. (2000) *Proc. Natl. Acad. Sci. USA* **97**, 2641–2645.
25. Martin, R. A. (1986) *Paleobiology* **12**, 370–382.
26. Bloch, J. I., Rose, K. D. & Gingerich, P. D. (1998) *J. Mammal* **79**, 804–827.
27. Damuth, J. & MacFadden, B. J. (1990) *Body Size in Mammalian Paleobiology, Estimation and Biological Implications* (Cambridge Univ. Press, Cambridge, U.K.).
28. Smith, B. H. (1992) *Evol. Anthropol.* **1,** 134–142.
29. Smith, B. H. & Tompkins, R. L. (1995) *Annu. Rev. Anthropol.* **24**, 257–279.
30. Nishikawa, S. (1992) *Anat. Rec.* **232**, 466–478.
31. Pfretzschner, H. U. (1994) *Palaeontographica A* **234**, 1–88.
32. Stefen, C. (1997) in *Tooth Enamel Microstructure*, eds. von Koenigswald W. & Sander, P. M. (Balkema, Rotterdam), pp. 63–83.
33. Clemens, W. A. (1997) in *Tooth Enamel Microstructure*, eds. von Koenigswald, W. & Sander, P. M. (Balkema, Rotterdam), pp. 85–112.

34. Wood, C. B., Dumont, E. R. & Crompton, A. W. (1999) *J. Mamm. Evol.* **6,** 177–214.
35. Dean, M. C. (1987) *J. Hum. Evol.* **16,** 157–172.
36. Dean, M. C. & Beynon, A. D. (1991) *Am. J. Phys. Anthropol.* **86,** 215–228.
37. Koch, P. L., Zachos, J. C. & Dettman, D. L. (1995) *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **115,** 61–89.
38. Fricke, H. C., Clyde, W. C., O'Neil, J. R. & Gingerich, P. D. (1998) *Earth Planet. Sci. Lett.* **160,** 193–208.
39. Gingerich, P. D. (1983) *Science* **222,** 159–161.
40. Gingerich, P. D. & Gunnell, G. F. (1995) *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **115,** 227–247.
41. Hadly, E. A., Kohn, M. H., Leonard, J. A. & Wayne, R. K. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 6893–6896.
42. Fortelius, M. (1985) *Acta Zool. Fennica.* **180,** 1–76.
43. Hunter, J. P. & Jernvall, J. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 10718–10722.

COMMENTARY